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FURTHER STUDIES ON THE SUBTERRANEAN ALGAL FLORA OF THE MISSOURI BOTANICAL GARDEN

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Historical Introduction

Although it has long been recognized that the surface of soil forms a very suitable habitat for many algae, especially the *Cyanophyceae*, it is only in comparatively recent times that investigators have realized that these small plants may possibly play an important part in the biology of the soil. The history of the investigation of soil algae is therefore of comparatively recent date, and, as contrasted with that of the other soil organisms, such as bacteria or protozoa, our knowledge of the algal flora of soil is very imperfect. It seems possible that the presence of these autotrophic plants in the soil may be of great importance, and that their physiological processes may greatly influence soil conditions and also have important effects on the soil as a medium of growth for the other soil organisms. However, any such influence exerted by soil algae is yet to be proved, for we are still in complete ignorance on this matter.

The literature bearing on the problem of soil algae, either directly or indirectly, is nevertheless very extensive although confined to two or three main lines of investigation, namely, the

systematic study of soil algae, their relation to nitrogen, whether or not they are capable of fixing atmospheric nitrogen, and lastly, an immense amount of work has been done on the physiology of algae, their relation to light and organic food substances, which is very important in the soil algae question. Since no very complete review of this literature exists, it is desirable that a summary of the more important papers be made here. The three phases of the literature mentioned above will be taken up separately.

The Systematic Study of Soil Algae

Although the systematic examination of the algae occurring in or on the surface of the soil was not undertaken for many years after the development of the soil algae problem, it will possibly be best to begin with this phase in order that some idea may be obtained of the nature of the organisms concerned.

The first extensive work relating to the soil algal flora was published by Esmarch ('10) and dealt with samples taken chiefly from soil in German African colonies. Esmarch's method of culturing his soil samples was such as to favor the growth of *Cyanophyceae*, and he confined himself almost exclusively to the consideration of these forms. The work first published by him was not undertaken primarily as an investigation of soil algae, but rather to increase the knowledge of the distribution of the *Cyanophyceae*. The soil samples fell into two main groups as regards the depth from which they were taken, namely, 1–25 cm. and 25–50 cm. Altogether, between 30 and 40 species were identified, chiefly species of the *Oscillatoriaceae* and *Nostocaceae*. In considering his data Esmarch came to the conclusion that the majority of the species occurred in the samples from the upper 25 cm., and that a greater proportion of the samples from the lower depths produced no growth. He was stimulated by these results to take up in greater detail the study of soil algae, with the result that in 1914 he published a second paper dealing with extensive investigations of the soil algae in Germany. Esmarch's second paper deals chiefly with the relation of the soil algal flora to depth and to cultivation. With regard to cultivation, surface samples taken from cultivated soils almost always produced a

good growth of algae, whereas very few samples from uncultivated soils produced algae except damp sandy soil from the shores of the river or lakes. At 10–50 cm. below the surface no algae could be obtained from soil samples from uncultivated soils, while samples from a similar depth in cultivated soils frequently produced a growth, though fewer species were represented than at the surface. Esmarch felt that since in cultivated soils the lower strata contained approximately the same species as the surface, with the exception that fewer species were present, the relation between the two levels might be such that the lower layers derive their flora directly from the surface in the operations of cultivation, such as ploughing or by the action of earthworms or of seepage water. Esmarch experimented also on the effect of prolonged darkness on some of the algae isolated by him from the soil, but was unable to produce any conclusive proof that they are able to persist for any great length of time in total darkness.

Robbins ('12), investigating the algal flora of the surface and first few inches of soils in Colorado with special reference to their extraordinary nitrogen-fixing capacity, in spite of their low content of organic matter, recognized about 21 species of algae, chiefly *Cyanophyceae*, but including two *Chlorophyceae* and a diatom. He believed that the abundant blue-green algae formed a source of carbohydrate food for the nitrogen-fixing organism, *Azotobacter*, and that for this reason the bacteria were able to flourish in quantity in spite of the low organic content of the soil.

Petersen's work in 1915 deals chiefly with diatoms growing on the surface of the soil, but some attention is also given to *Chlorophyceae*, although the *Cyanophyceae* are entirely ignored.

Bristol ('19a) gives an interesting account of algae obtained from soil samples preserved in an air-dry condition for many years and extended our knowledge not only of the degree of resistance to desiccation of these forms, but also the range of species of the soil algal flora. Two years later the same author (Bristol, '21) published a more extensive soil flora. From an investigation of 50 soil samples taken from the surface 6 inches of soil she identified 20 species of *Chlorophyceae*, 24 *Cyanophyceae*, and 20 diatoms. This must be regarded as the most complete algal flora of the soil published so far. The *Chlorophyceae* included chiefly unicellular

forms, although *Bumilleria exilis* and *Ulothrix subtilis* were almost constantly present; the *Cyanophyceae* were largely species of *Nostoc*, *Anabaena*, and *Phormidium*, and the diatoms, *Navicula* and *Nitzschia* spp.

Moore and Karrer ('19), taking up again the investigation of the lower strata of the soil as initiated by Esmarch, established the existence of diatoms and other algae at depths up to 100 cm. below the surface of the ground, the greatest depth at which such organisms had been recorded. The interesting fact was discovered that a unicellular green alga which they identified as *Protoderma viride*[1] is constantly present as far down as 1 m. below the surface. Repeating Esmarch's subterranean culture method, in a slightly varied form, they found that this algae could live and remain green when sunk into the ground for a period of 4–5 months.

The Relation to Atmospheric Nitrogen

By far the greatest amount of work done so far has been concentrated on the relation of the algae to nitrogen and especially to atmospheric nitrogen.

Even in the early fifties following the work of Boussingault and his contemporaries on the nitrogen relations of the higher plants, Laurent ('54) and Morren ('54) came independently to the conclusion that lower organisms, including protozoa and algae, are unable to make use of atmospheric nitrogen, but pass into a resting condition when the supply of combined nitrogen becomes depleted in the medium in which they are living.

For more than 30 years no further work seems to have been done until the opposite opinion was put forward by Frank ('88), who discovered that if soil is kept moist with distilled water there is an increase in the nitrogen content after standing for several months. Frank's attention was drawn to the fact that the increase in nitrogen was not present in the form of nitrates, but in an organic form. Furthermore, on the surface of the soil he noticed that a mat of algae had developed, including *Oscillatoria* spp., *Chlorococcum humicola*, and others. Frank thus came to the conclusion that atmospheric nitrogen is transformed into nitrates

[1] This now appears to be identical with *Chlorococcum humicola* (Näg.) Rab. as recorded by Bristol ('19b, '21) and others.

by the algae in the soil, and that these are later elaborated into a complex organic form.

In the same year Gautier and Drouin ('88), working with soils containing nitrogen only in an ammoniacal form, found that such soils, if poor in organic matter, show a lower nitrogen content after standing, but that a higher nitrogen content will result if much organic matter is present. In all cases, however, irrespective of the organic content of the soil, the ammoniacal nitrogen decreases and the organic nitrogen increases, the latter in direct proportion to the strength of the algal mat which develops on standing. These workers therefore drew the somewhat original conclusion that the algae are important as conservers of ammoniacal nitrogen, whose escape from the soil they prevent, rather than as fixers of atmospheric nitrogen. They thought that the escaping ammoniacal nitrogen is absorbed by the algae and transformed into a more stable organic form.

In the following year Frank ('89) put forward what he considered to be direct proof of the nitrogen-fixing power of the lower algae by demonstrating that whereas a substantial increase in nitrogen content takes place if soil is kept moist and exposed to daylight, no increase takes place in the same soil if it is kept in the dark, or sterilized. Either of these last two treatments prevents the growth of algae, while the darkness, although excluding the algae, permits growth of bacteria. Frank believed, therefore, that the increase of nitrogen taking place in the light was due entirely to the growth of algae.

The work of Schloesing and Laurent ('92) lent further support to Frank's idea. These workers used poor subsoil and sand for their substratum, and inoculated with impure suspensions of algae, adding also a small sample of ordinary soil extract. They kept their soil in a confined atmosphere and had an arrangement for determining the actual change in the gaseous nitrogen contained in that atmosphere. In this way they were able to check up the loss of gaseous nitrogen with the amount of nitrogen increase in the soil. With *Nostoc punctiforme* and *Cylindrospermum majus* they found a substantial increase in the nitrogen content of the soil, and they also established the fact that there is a greater increase of nitrogen in the upper layer of the soil con-

taining the algae than lower down. While realizing that bacteria possibly play a part in the increase of nitrogen, they believed that the algae, being present in larger numbers, are responsible in a greater measure for the fixation of atmospheric nitrogen which occurs.

In 1893, Koch and Kossowitsch kept sand cultures inoculated with a suspension of algae in daylight, and similar cultures as controls in darkness. Their experiments served to confirm the conclusions of Frank that in the presence of light active fixation of atmospheric nitrogen takes place, but that if the growth of algae is prevented by absence of light no fixation occurs.

Since this time there have been repeated investigations of the activities of algae in association with soil bacteria with special reference to nitrogen relations. One of the most energetic workers in this line was Bouilhac. In 1896, claiming to have isolated in pure culture certain algae, this worker found that *Nostoc punctiforme*, *Schizothrix lardacea*, and *Ulothrix flaccida*, when free from bacteria, were quite unable to live in a solution containing no nitrogen. *Nostoc punctiforme* could live perfectly well under these conditions provided that a drop of soil extract containing soil bacteria was added, and, moreover, the solution would show a decided increase in nitrogen content, showing that fixation of atmospheric nitrogen had taken place. The other two species, however, were incapable of living under similar conditions. Later, in 1897 and 1898, he showed that *Nostoc punctiforme* could live in association with soil bacteria in the absence of combined nitrogen, and also in darkness, provided that glucose is supplied. Without glucose no growth takes place in darkness. A very interesting experiment was recorded by the same worker in 1903, in which he showed that algae and soil bacteria, inoculated together into sterile sand with mineral nutrients devoid of combined nitrogen, are capable of fixing enough atmospheric nitrogen to support the growth of higher plants.

The idea of a symbiotic relationship between algae and soil bacteria has since gradually grown in popularity. Reinke ('03b) believes that *Volvox* colonies are ordinarily infested with the nitrogen-fixing organism *Azotobacter*, that the bacteria obtain some carbohydrate food from the gelatinous matrix of the colony,

and in return give up some combined nitrogen, in which they are very rich. In a somewhat earlier paper the same author (Reinke, '03a) extends this idea to marine algae, and expresses the probability that here also the algae are dependent upon *Azotobacter* for their nitrogen supply, and that the bacteria live embedded in the gelatinous surface of the algae.

Fischer ('04) asserts that there is a similar symbiosis between a terrestrial species of *Oscillatoria* and *Azotobacter*, and that even when the bacteria are not readily visible they can be made to increase rapidly by culture in 1 per cent mannite solution. It is interesting to note that he was unable to obtain the bacteria from *Hormidium parietinum* and *Pleurococcus vulgaris* inhabiting the bark of trees.

In opposition to all the work enumerated above and many other similar investigations too numerous to mention, all dealing with impure cultures or with algae and bacteria in mixed culture, indicating an increase in nitrogen content of the culture medium which was sometimes erroneously attributed to the algae themselves, is a group of articles dealing with experiments on algae in pure culture, free from bacteria, which give more conclusive evidence of the relation of algae to atmospheric nitrogen.

The earliest of these workers with pure cultures was Kossowitsch ('94) who isolated an alga from soil and proved quantitatively that no fixation of nitrogen could be demonstrated in the case of the pure alga, but that if impure cultures containing bacteria are used considerable fixation takes place. Kossowitsch realized the possibility of a symbiotic relationship.

Krüger and Schneidewind ('00) carried out very extensive experiments from which they drew similar conclusions. Working with about 8 species of *Stichococcus*, as many of *Chlorella*, and about 6 species of *Chlorothecium*, they proved that not one of these is able to live in a solution containing no combined nitrogen, and that even when growing vigorously there is no fixation of atmospheric nitrogen. They attribute the increased fixation in mixed cultures to the beneficial action of the symbiotic relation between the algae and bacteria rather than to the activity of the algae themselves.

Charpentier ('03), working with *Cystococcus humicola*,[1] also came to the conclusion that in pure culture this species is incapable of fixing atmospheric nitrogen.

Heinze ('06), working with a species of *Nostoc*, claims to have proved that fixation of nitrogen occurred in this species, but his alga was slightly infected with a species of *Streptothrix*. After isolating the fungus Heinze proved that this, by itself, was unable to fix atmospheric nitrogen, whereas the alga with its slight infestation showed a decided fixation. Heinze thus considered it proven that the fungus played no part in the nitrogen fixation, which he thought was due entirely to the alga. Since this worker confesses that he was unable to free his alga from the fungus, it is possible that bacteria were also present in the culture.

Schramm ('14), who gives a very complete survey of all the earlier work on the subject, working with 7 diverse species of soil algae in pure culture, proved that when no combined nitrogen was provided, not one of these algae was able to grow. This seems to indicate that they are incapable of making use of atmospheric nitrogen.

Nakano ('17), in the course of his paper, gives a more thorough investigation of the symbiosis between *Azotobacter* and algae than any other worker. He used species of *Chlorella*, *Scenedesmus*, etc., in pure culture, and also isolated and named from soil a pure strain of *Azotobacter*. Using a solution containing no combined nitrogen and .5 per cent glucose, Nakano found that the algae were unable to produce any growth, and moreover fixed practically no nitrogen when grown in pure culture in such a solution. *Azotobacter*, however, grew well under the same conditions and fixed atmospheric nitrogen abundantly. Mixing the pure *Azotobacter* and the pure algae in the same culture solution, a good growth of algae resulted, and in addition the amount of nitrogen fixed by the mixed culture of *Azotobacter* and algae was 20 per cent higher than by the pure strain of *Azotobacter* alone. This clearly proves the beneficial effect of the association of the two organisms. In further discussion concerning the more exact nature of the symbiosis, Nakano comes to the conclusion that the algae live at the expense of nitrogenous compounds derived from

[1] Probably identical with *Chlorococcum humicola* (Näg.) Rabenh.

the *Azotobacter*, which they obtain after the death and autolysis of the bacterial cells. A sterilized culture of *Azotobacter* is not capable of furnishing the nitrogen necessary for algal growth, since the nitrogen is unavailable in a complex organic food, and enzyme action is destroyed. Debating on the conditions in nature, Nakano is inclined to believe that the symbiosis is not of any real importance to the algae, for he argues that whereas many thousands of *Azotobacter* cells are necessary to supply nitrogen for a single algal cell, one never sees algae thus infested in nature, and he is very doubtful whether *Azotobacter* could supply the nitrogen required by the larger seaweeds. In explaining the beneficial action of the algae on the nitrogen-fixing capacity of *Azotobacter*, Nakano thinks that possibly the oxygen evolved by the algae during photosynthesis is responsible for this. *Azotobacter* is more active if grown in thin layers than in thick layers and thus it seems to have a high oxygen requirement. The presence of algae might therefore be helpful so long as photosynthesis is going on.

In the past few years very few workers have claimed that algae by themselves are capable of fixing atmospheric nitrogen. Benjamin Moore and Webster ('20), and later Benjamin Moore, Whitley, and Webster ('21) have performed certain experiments and claim that nitrogen fixation is possible in both freshwater and marine algae. They made no attempt, however, to free their cultures from bacteria, and moreover they were apparently working in complete ignorance of the extensive literature on the subject and the results of the many workers who had preceded them. They were apparently also unaware of the fact, long proven at that time, that a symbiotic relationship exists between nitrogen-fixing bacteria and algae. Their results cannot therefore be considered to add anything to our knowledge of the relation of green algae to atmospheric nitrogen, although their philosophic discussions are interesting.

Wann ('21), on the other hand, used only pure cultures, and he also claims that nitrogen fixation is possible under certain conditions in green algae. Using 7 different species, he supplied his algae with nitrogen in several forms, in organic combination or as ammonium or calcium salts, in series with and without glucose.

Analysis was made of the media before the experiment, and of the culture solutions at the conclusion, by the Gunning-Kjeldahl method, and it was found that where the nitrogen was provided in the form of nitrate and in the presence of glucose, there was an increase in nitrogen content amounting to as much as 54 per cent. The author therefore concludes that the algae had fixed atmospheric nitrogen to that amount. Wann's work occupies a very isolated position, being the only case in which fixation of atmospheric nitrogen by green algae in pure culture has been claimed.

Muenscher ('23), working on the nitrogen metabolism in *Chlorella*, states incidentally that his results do not conform with those of Wann and that he always recovered the same amount of nitrogen after the experiment as was provided at the beginning. He was unable to demonstrate nitrogen fixation in this species.

Wann's work has been criticized by Bristol and Page ('23). These workers have carefully repeated Wann's experiments, and find that there is no fixation of atmospheric nitrogen by the algae used by them. They claim to have found the weak point in Wann's work to be the method of analysis when nitrates are present. They seem to prove their point fairly conclusively, and as far as one can judge from the evidence at present it seems quite likely that Wann's results will have to be held in abeyance until an explanation can be given of why his results are at variance with the work of other investigators.

Summarizing our knowledge of the relation of algae to nitrogen, it seems to be fairly well established that green algae in pure culture, free from bacteria, are unable to fix atmospheric nitrogen, but that when cultivated in association with *Azotobacter*, their presence seems to be beneficial, so that the capacity of the bacteria for fixing nitrogen is stimulated. The relation between the two organisms is probably of a symbiotic nature, the bacteria deriving carbohydrate food material from the gelatinous sheaths of the algae, and the algae thriving on the nitrogenous material provided by the bacteria.

THE RELATION TO LIGHT AND CARBON

In the consideration of the peculiar conditions in which algae must live if they are present beneath the surface of the soil in an

active condition, their relation to darkness and capacity for saprophytic nutrition must be important. According to Bristol, the number of algae in the soil at 4 inches below the surface is nearly as great as in the surface inch. Furthermore, the quantity is far greater than usually supposed. "Taking 100,000 as a rough estimate of the number of algae per gram of manured soil in a given sample and assuming the cells to be spherical and of average diameter, 10 μ, it has been calculated that the volume of algal protoplasm present was at least three times that of the bacteria, though only one-third that of the protozoa." [Russell, The Micro-Organic Population of the Soil, p. 110. 1923.]

Such great numbers of algae living at the depths at which they are found must be in complete darkness and under conditions which render photosynthesis impossible. A considerable amount of work has been done on the physiology of the algae, and the question of their capacity for saprophytic nutrition in the absence of light has received ample attention. There is so much literature on the subject that only a few of the cases in which saprophytic nutrition has been demonstrated can be mentioned.

Radais ('00), working on *Chlorella vulgaris*, found that his cultures remained green in darkness and also that when grown on potato malt agar, growth was equally good in light and in darkness, while Grintzesco ('03), working with the same organism, obtained exactly the same results, and even states that with 2 per cent glucose, growth in total darkness may be much better than in the light. Artari ('06) and Kufferath ('13) also support these statements.

Charpentier ('02, '03) found that *Cystococcus humicola*[1] grew and remained green in darkness, but that the yield was much greater in the light, a weight of 330 mgms. resulting in the light as against 27 mgms. in darkness. Artari ('02) also found that the same alga produced normal healthy growths if grown in darkness with 1 per cent mannite, lactose, glucose, levulose, or canesugar.

Treboux ('05) has shown that other substances than carbohydrates may be used by algae as a source of carbon in the absence of light, for he obtained growth with several species of algae in

[1] Probably *Chlorococcum humicola* (Näg.) Rabenh.

darkness, using the potassium salts of various organic acids and also the amino derivatives, such as glycocoll, alanin, leucin, and asparagin. With the acids, the simplest compounds seemed to be the most easily assimilated, and a concentration of .25 per cent acetic acid was used by practically all the 40 species of algae investigated.

Chlamydomonas Ehrenbergii, according to Artari ('14), is able to use glucose in darkness, for if glucose is present the yield in the absence of light is 20–25 times greater than if no sugar is given. However, the growth is at no time so good as when autotrophic nutrition is permitted.

Dangeard ('21) records that he has grown *Scenedesmus actatus* in darkness for 8 years. One per cent glucose and .8 per cent peptone were provided, and frequent transfers to new media were made. After being subjected to darkness and prevented from exercising its photosynthetic function for so long, exposure to light resulted in the evolution of oxygen in 5 hours. This is very interesting in connection with the soil algae question.

The above work, confined to species of the *Chlorophyceae*, is altogether in favor of the possibility of heterotrophic nutrition in darkness. Not so much work of this kind has been done on the *Cyanophyceae*, and the evidence is not nearly as conclusive. Bouilhac ('97) found that *Nostoc punctiforme* would grow in darkness provided that glucose was present. His experiment, however, is complicated by the fact that he was using a mixed culture with soil bacteria. Pringsheim ('13) isolated several species of *Nostoc* and *Oscillatoria*, but found that the addition of organic matter to the cultures produced deleterious effects if used in large quantities, and that the stimulating action of small amounts was never very striking. He was unable to demonstrate that saprophytic nutrition with organic food was possible in the dark.

CULTURAL METHODS AND RESULTS

The culture vessels were prepared in essentially the same way as described in an earlier paper (Moore and Karrer, '19) except that the sand was well washed before being used and the culture solution was full strength. It was not felt necessary to slant the

bottles, since the sides of the bottle served as a suitable substratum for forms not requiring aquatic conditions. All the samples were taken from excavations in the Missouri Botanical Garden in localities where the soil had not been disturbed for at least twenty-five years, so that the results obtained should be regarded as pertaining to uncultivated rather than to cultivated soils. For the most part the several localities had been covered with a dense turf. The depth from which the samples were taken was noted at the time, and every precaution observed to prevent contamination of the samples with soil from a different level. In the case of the first 3 series of cultures the culture vessels were inoculated in the garden with an unknown amount of soil, but in the fourth series the samples were taken in sterile bottles, and inoculation was performed in the laboratory with weighed amounts of soil. In order to prove that infection from the air did not take place during the weighing of the inoculum, a sterile control culture was left exposed to the air of the laboratory for 36 hours during the weighing of the soil, but no growth of algae resulted from this exposure. The algae developing in the cultures can therefore be assumed to have developed from the soil with which they were inoculated.

The first 3 series of cultures were inoculated with soil of different levels down to 4 or 5 feet, but in the last series the samples were taken as deep as 9–10 feet.

The results recorded here can be considered as amplifying the data given in the earlier paper (Moore and Karrer, '19), not only in extending our knowledge of the depth at which algae occur in the soil but also in giving an idea of the variety of the subterranean flora. Although by no means as luxuriant as at the surface (as investigated by Bristol, '19a, '21), the flora nevertheless includes a far greater number of species, especially *Chlorophyceae*, than one would expect in subterranean conditions.

The species which is almost universally present is *Chlorococcum humicola*, recorded as *Protoderma viride* in the earlier paper. It should be noted that Bristol ('19b, '21) has obtained this alga from the Malay States, and has also found it usually present in English soils. It therefore seems likely that this species is universally present in all soils. In the present cultures it appeared in almost

every case where a rough inoculum (about 10 gms.) of soil down to 3 feet was used, and it will probably occur in samples as small as .1–.2 gms.[1] Below 3 feet, however, its occurrence is so uncertain that one cannot be sure of obtaining it unless more than 5 gms. of inoculum are used.

Chlorococcum humicola is accompanied by a number of other green algae, which seem to inhabit the soil in somewhat smaller numbers than this dominant species, so that some, but not all, may occur along with *Chlorococcum* in most of the cultures. Below 3 feet it not infrequently happens that one or other of these species may be obtained in a unialgal culture, a circumstance which aided considerably in their identification as distinct species.

Whereas *Chlorococcum humicola* occurs with sufficient constancy for it to be possible for us to come to some conclusions regarding its numerical distribution in the lower layers of the soil, most of the other species seem to be very uneven in their distribution and to occur without any regularity, so that it is impossible to make any definite statements concerning their numbers in the soil.

It is noteworthy that these accessory species do not correspond very closely to the list of *Chlorophyceae* enumerated by Bristol ('21) as accompanying *Chlorococcum humicola* in the English soils. One species which is given by her, namely, *Chlorochytrium paradoxum*, is, however, more constantly present in the Missouri samples than in England. Apart from this, there is almost no conformity between the two lists, and one is forced to the conclusion that the subterranean flora is probably as variable as the surface flora.

The most interesting species isolated from the Missouri soils and not present in Miss Bristol's list is *Protosiphon botryoides*, nearly always present in our cultures (and possibly "*Cladophora* sp." in Moore and Karrer, '19). This species is not native in England and is therefore not likely to occur in the subaerial flora. The frequent occurrence of *Chlorella* in our cultures, and its absence from the British list is, on the other hand, somewhat surprising, and one cannot help thinking that possibly Miss Bristol

[1] The weight of inoculum must only be regarded as approximate, since the moisture, which varied in samples from different levels, was not taken into account.

overlooked it, or mistook it for small cells of *Chlorococcum humicola*.

Botrydiopsis arhiza is another species which is probably more often present than is apparent from the accompanying lists, since isolated cells are easily overlooked. As regards the species of *Chlamydomonas*, it is possible that Miss Bristol has included at least one species in her stages in the life history of *Chlorococcum humicola* ('19b). The form figured by her in pl. 18, figs. 27, 28, is very similar to the alga recorded here as Species B. *Chlorococcum humicola* has been isolated by one of us in pure culture, and a palmelloid stage has never been observed to occur. Species B has also been isolated, and it always retains its characters both on solid and liquid media.

Species of *Cyanophyceae* and diatoms are notably much fewer than recorded in Miss Bristol's surface flora, and the *Cyanophyceae* only occurred in dominating numbers as a rule in the upper 18 inches of soil. On the other hand, the development of *Lyngbya subtilis*, 2½ feet down, in Series A, is suggestive of the possibility that in the method of culture used the development of *Chlorophyceae* was perhaps unduly favored. The obtaining of a pure culture of *Oscillatoria amphibia* from a depth of 8 feet 2 inches in Series D is likewise a very interesting fact, which was considered at first as being due to a chance contamination from a different level. However, since the greatest precautions were taken during the collecting of the samples and the inoculation of the cultures, and, moreover, since isolated cultures of different species were obtained from samples of soil taken from 5 to 9 feet in about 8 different cultures, it seems unlikely that foreign infection was responsible for all 8 cultures. This is especially true since with one or two exceptions the constituents in these cultures are not at all common in the cultures from higher levels, and represent in some cases the only record of that particular species. If the development of these cultures from lower levels is due to infection with soil from a higher level, the species most commonly occurring there, namely, *Chlorococcum humicola*, would be expected to appear.

The diatoms are not fully recorded here for the first 18 inches, since a few other species sometimes occurred in the uppermost

samples taken, but in too small numbers for the cleaning up of a sample for specific determination to be a successful operation. The records for the lower depths can be considered fairly accurate. The record of *Navicula atemoides* in pure culture at a depth of 5 feet 5 inches is extremely interesting, and this is the species most constantly present in cultures from the lower strata.

In identifying the species the greatest difficulty was experienced in deciding between *Chlorochytrium paradoxum* and *Protosiphon botryoides*, and it was often impossible to determine whether one or both of the species were present. *Protosiphon*, after a length of time in culture, proceeds to form large resting aplanospores which may be released from the old mother cell-wall and remain for a long period as orange-colored cysts. *Chlorochytrium paradoxum* also forms similar large cysts which cannot be distinguished from those of *Protosiphon*. The records for these two species are therefore somewhat uncertain.

The succession of species in the cultures was very interesting, for with age, species originally dominating would fade away and give place to others. *Protosiphon* begins to lose its typical form after about 6 months' culture, probably because an aquatic habitat is not normal for it. The most interesting case, however, is that of the alga recorded as Species A, which was never observed until a culture reached the age of 8–12 months, except in the culture 42C where it occurred as a unialgal culture and formed a recognizable but feeble growth in 3 months. Probably high organic food requirement is responsible for these facts.

It should be noted that series D produced a large number of species which were not observed at all in the three earlier series. Possibly this is due to the fact that a greater proportion of unialgal cultures occurred in this series. These rarer species may have been present in the other series, but were not conspicuous because of the greater mass of other forms, or again the difference may be due to a local variation in the flora.

Notes on the Species Observed

CYANOPHYCEAE

Nostoc commune Vauch.

Forti in De Toni, Syll. Alg. **5:** 404. 1907.

This species occurred in a well-developed form in several cultures, all above 1 foot 4 inches. The trichomes were about 5 μ in diameter, with heterocysts very slightly larger than, or equal in size to, the vegetative cells. Spores were abundant in long chains and measured about 6 × 8 μ.

Nostoc muscorum Ag.

Forti in De Toni, Syll. Alg. **5:** 400. 1907.

This species was identified in only one culture, from a depth of 6 inches. The spores were somewhat immature, but the other characters seemed sufficient to identify it.

Nostoc comminutum Kütz.

Forti in De Toni, Syll. Alg. **5:** 393. 1907.

A pure culture of an alga corresponding to this species was obtained from soil at a depth of 3 feet 6 inches. The colonies were small and distinct from each other, consisting of trichomes 3–3.5 μ in diameter, closely convoluted. Heterocysts 6.5 μ in diameter were observed, but spores were wanting. The occurrence of a blue-green alga at such a depth is somewhat surprising.

Phormidium tenue (Menegh.) Gom.

Forti in De Toni, Syll. Alg. **5:** 227. 1907.

This species was identified in only one culture, at a depth of 1 foot. It was quite typical.

Phormidium molle (Kütz.) Gom.

Forti in De Toni, Syll. Alg. **5:** 219. 1907.

In the same culture as the preceding species was a small quantity of a very moniliform *Phormidium* with trichomes 3.5 μ in diameter, which seemed to correspond fairly well with the description given for this species.

Lyngbya subtilis West

Forti in De Toni, Syll. Alg. **5**: 285. 1907.

In a culture of soil from a depth of 2½ feet there developed after a time a quantity of a very slender blue-green alga with a distinct sheath. The filaments were about 1.5 μ in diameter, and the trichomes themselves barely 1 μ. The alga seemed nearest to *Lyngbya subtilis* West.

Oscillatoria amphibia Ag.

Forti in De Toni, Syll. Alg. **5**: 169. 1907.

The development of a pure culture of this species from soil of more than 8 feet depth was a very surprising phenomenon. The alga was in every way normal and typical.

BACILLARIEAE

Navicula atemoides Grun.

Van Heurck, Diat. 227. *pl. 5, f. 230.* 1899.

This was the most general of all diatoms, and was a general constituent of the flora from the surface 6 inches to a depth of 5 feet 5 inches, which is the lowest record obtained for it.

Navicula mutica Kütz.

Van Heurck, Diat. 206. *pl. 4, f. 167.* 1899.

This species was not quite as frequent as the preceding one, but nevertheless has been found at a depth of 4 feet.

Hantzschia amphioxys (Ehr.) Grun.

Van Heurck, Diat. 381. *pl. 15, f. 483b.* 1899.

This is one of the less frequent diatoms.

Nitzschia palea (Kütz.) W. Sm.

Van Heurck, Diat. 401. *pl. 17, f. 554.* 1899.

Rather more frequent than the preceding species and observed below 4 feet.

CHLOROPHYCEAE

Chlorococcum humicola (Näg.) Rab.

Bristol in Jour. Linn. Soc. Bot. **44**: 473. 1919.

This alga developed in nearly every culture down to 3 feet, and the lowest record is 5 feet 5 inches. As will be seen from table IV, series D, .01 gm. of soil at 12 inches depth gave a good growth of *Chlorococcum*. It can therefore be assumed that at this depth there are at least 100 individuals of the alga to the gram. At 3 feet depth, however, they may be as sparse as one individual in 2 gms., and at 4 feet 2 inches rarer than one in 5 gms. The alga reproduced freely by both zoogonidia and aplanospores, and the vegetative cells readily formed orange resting cysts when conditions became unfavorable for vegetative growth. There is some doubt as to whether the large cells mentioned by Bristol ('19b) really belong to this species. They possibly belong to *Chlorochytrium*. Furthermore, the palmelloid stage mentioned and figured by Bristol probably belongs to a species of *Chlamydomonas*, Species B of this work (*vide supra*).

Chlorochytrium paradoxum (Klebs) West

Bristol in Jour. Linn. Soc. Bot. **45**: 8. 1920.

This species was a fairly frequent constituent, though it was often difficult to decide for certain reasons whether it was really present or not, especially when *Protosiphon botryoides* was also present (*vide supra*). It usually occurred in the form of large olive-green cells or bright orange resting cysts, reaching a diameter of 100 μ. There were rarely any of the smaller cells showing the characteristic cytological structure of the genus, and the only way in which the identity of the cysts was suspected was in following the development of the zoogonidia into the characteristic vegetative cells. Large aplanosporangia with a few comparatively large cysts of unequal size were sometimes observed, all in a resting condition. The thickness of the wall of the aplanosporangium might be as much as 7–10 μ.

Protosiphon botryoides (Kütz.) Klebs

Klebs, Bed. Fortpflanz. 169–222. *pl. 1, f. 1–16*. 1896.

This species was a very frequent constituent of the cultures, being present in practically all samples in series B and C. Very often it occurred in great abundance, so that it was probably present in considerable quantity in the soil. In the cultures the alga was not always typical in form because of the aquatic conditions but it was always easily recognized. Reproduction by aplanospores of varying size was frequent, and the production of gametes, their conjugation, and the formation of the tiny star-like zygotes figured by Klebs (*loc. cit., pl. 1, f. 16*) were also observed. Sometimes the aplanospores were transformed into large, orange, thick-walled resting cysts. The lowest depth from which the species has been obtained is 4 feet.

Chlorella spp.

The genus *Chlorella* was represented fairly constantly in the cultures, and it seems quite possible that more than one form occurs. The specimens isolated from a certain culture measured uniformly 3–5 μ in diameter, whereas in other cultures they were only 2–3 μ in diameter. This indicates that possibly two forms occur in the culture, but in the records no distinction is made between the two. *Chlorella* has been observed as far down as 5 feet.

Trochiscia reticularis (Reinsch) Hansg.

West, Brit. Freshw. Alg. *f. 82K*. 1904.

This species occurred in 3 of the 4 series of cultures, and in the last series, D, was almost as constant and as abundant a constituent as *Chlorococcum humicola* itself, the indications being that there are more than 100 specimens per gram at a depth of 12 inches. It must be noted, however, that whereas .01 gm. of soil from this level produced individuals of *Trochiscia*, 5 gms. from the same level failed to produce any specimens. The distribution of this species therefore seems to be very uneven. The specimens varied in size from 10 to 20 μ in diameter, and were sometimes oblong rather than spherical in outline, with the dimensions 12 × 8 μ. The lowest record for the species is 3 feet 9 inches.

Trochiscia sp.[1]

?Acanthococcus sp., Reinsch, Ber. d. deut. bot. Ges. 4: 243. *pl. 12, f. 18.* 1886.

This species was observed only in a single culture, at a depth of 3 feet 9 inches. It was sharply distinguished from *Trochiscia reticularis*, which was the more common species of the genus, by its short, blunt, and rounded projections. The specimens varied in size from 14 to 18 μ in diameter and seemed nearest to the form recorded by Reinsch as *Acanthococcus* sp.

Dactylococcus sp.

Cells of this form were observed only in two cultures. They were stout and spindle-shaped with slightly acute apices, and measured about 12 × 8 μ. A pyrenoid was distinctly present, and reproduction was observed by the formation of 4 similar individuals within a mother cell, which might at this time reach the size of 12 × 16 μ. In general appearance the form was most suggestive of the *Dactylococcus* state of *Scenedesmus* figured by Grintzesco ('03, p. 217), but what it really is was not decided. Bristol ('21, p. 74) records a species of *Dactylococcus* from English soils, but there can be no confusion between the form observed here and her species.

Ulothrix subtilis Kütz.

West, Brit. Freshw. Alg. 76. *f. 20 C-F.* 1904.

This species is not at all a frequent constituent of the subterranean flora, although, according to Bristol ('21), it is almost universally present on the surface. In two of the series of cultures it did not occur at all, and its occurrence in the other two series was sporadic. The record at 3 feet in series D is somewhat surprising. It seems probable that for some reason this alga does not usually descend very far from the surface of the soil, possibly owing to the fact that its zoogonidia are not long motile. The filaments were 5–7.5 μ in diameter, and the cells almost as long as, or a little longer than, broad. There was some tendency for the filaments to break up into short lengths.

[1] Bristol ('21) records two species of *Trochiscia* from English soils, but neither seems to be identical with the forms observed here.

Stichococcus bacillaris Näg.

West, Brit. Freshw. Alg. 80. *f. 24A*. 1904.

This species does not seem to occur with any regularity, unless, as is quite possible, the tiny cells were often overlooked. It was observed in only 3 cultures, the lowest record being 2 feet 6 inches.

Stichococcus scopulinus Hazen

Hazen in Mem. Torr. Bot. Club **11**: 161. *pl. 22, f. 4–6*. 1902.

This alga was observed in only two of the series and never below a depth of 12 inches. The cells were 3–4 μ in diameter and 11–16 μ in length. The filaments were often of considerable length and showed little tendency to dissociate. It seems likely that the occurrence of this species in the subterranean flora is dependent upon its local distribution at the surface and that it probably never descends to any great depth.

Uronema confervicola Hazen

Collins, Green Alg. N. Am. 88. *f. 66*. 1909.

The occurrence of a few isolated filaments of this species in 2 cultures from a depth of 3 feet was indeed surprising. The filaments were about 4 μ in diameter and of great length. The cells were provided with 2 pyrenoids each, and the apical cell was typically acute. The species is probably not a regular inhabitant of the soil but only a chance form. In both cultures it was present in such small quantity that a month after it was first observed it had quite disappeared.

Monocilia viridis Gerneck

Gerneck, Beih. Bot. Centralbl. II. **21**: 263. *pl. 12, f. 77–84*. 1907.

A form closely resembling this alga described by Gerneck in structure and appearance was observed in 3 cultures of series A. In the culture from the lower level, 3½ feet, it was present in considerable quantity and for a time favored the dominant constituent of the culture. Later it disappeared entirely from both cultures and left no trace. In the culture in which it had been most abundant, a considerable quantity of a yellow-green alga

appeared which was identified as *Botrydiopsis arhiza* Borzi. In this connection it is interesting to note that Gerneck states that *Monocilia viridis*, after being cultured for about 2 months in a liquid medium, loses its branched and filamentous form and goes into a unicellular palmelloid state. According to Gerneck, the filamentous form can only be obtained from the palmelloid stage by cultivating on a solid medium such as agar. The alga identified as *Botrydiopsis arhiza* in these cultures has been grown on agar, however, and it always retained its unicellular form. If Gerneck's observations are correct, therefore, it would seem that *Monocilia viridis* and *Botrydiopsis arhiza* as observed in these cultures are distinct from each other. It may be that *Monocilia viridis* is a more constant inhabitant of the soil than would appear from these records, but that it is not often in a recognizable condition.

Botrydiopsis arhiza Borzi

Borzi, Studi Alg. **2:** 169. *pl. 12, 13.* 1895.

This is the most regular representative of the *Heterokontae* in the subterranean flora. It is rarely present, however, in great abundance, and is possibly often overlooked. When there is not too much competition with other species it multiplies rapidly, however, and may form an abundant growth. Reproduction by aplanospores was very common, and zoogonidia with only one visible cilium were also observed. On one occasion biciliate swarmspores, similar to the gametes figured by Borzi, were seen, but no conjugation took place. The lowest depth from which the alga was obtained was 4 feet.

Characiopsis minuta Borzi

Borzi, Studi Alg. **2:** 152. *pl. 14, f. 1–12.* 1895.

The occurrence of this species in a single culture was somewhat surprising. It was present in considerable quantity in a sample taken from a depth of 2 feet 3 inches and only differed from the typical form in its slightly smaller size. The finding of this species seems to indicate that the spores of many algae may occasionally find their way into the soil and suggests that the subterranean flora may prove, with increased investigation, to be

almost as rich in species as the surface flora, though not in numbers of individuals.

Species A

The form recorded under this heading is a very problematical one which, since swarmspores have not yet been observed, cannot be properly identified. It is probably a fairly constant inhabitant of soil, but evidently requires very special conditions for its development, for it only appears in old cultures. The cells float freely in the water, quite isolated from each other and without any tendency to adhere in colonies. They are usually oval-oblong, 10–28 μ long by 7–18 μ broad, though quite frequently they may be spherical with a diameter of 9–15 μ. In some instances a number of unusually large individuals may occur scattered among the smaller ones, either spherical or oval, and reaching a diameter of 40 μ.

The most striking feature of the alga is the presence of a bright red spot in the interior of the cell. This is obviously not a stigma, for it is much larger, reaching a diameter of 2–8 μ. The chloroplast is a small parietal plate which only covers part of the wall. There is neither a pyrenoid nor starch present, though oil is abundant. The systematic position of the alga is unknown.

Species B

This is most probably a species of *Chlamydomonas*, and was the most frequently encountered representative of the genus. It is in all probability more constantly present than is indicated by the tables, and, especially in the earliest examination of series A, was possibly very often mistaken for stages of *Chlorococcum humicola*. Complete isolation of the form has shown, however, that it is a distinct species. It usually occurred as oval cells embedded in a gelatinous stratum, and if present in any great quantity, or if pure, forms a soft gelatinous stratum similar to masses of *Tetraspora*. A slight change in temperature causes the green cells to acquire cilia and to swim out of the gelatinous matrix. The period of swarming is comparatively short, and the cells soon become quiescent again and secrete quantities of mucilage to form a large expanded colony as before. Multiplication takes place in the palmelloid stage by the division of individual cells,

usually into fours. The motile cells are oval in form, 7–12 μ long by 3–7.5 μ wide. They have a massive bell-shaped chromatophore which only leaves a minute rounded clear space at the apical end of the organism. There is a distinct pyrenoid, usually in the posterior region, but sometimes more or less lateral. The stigma is distinct and somewhat elongated, lying in the apical region of the cell. The two cilia are about equal to the body of the organism in length. Bristol ('21) does not record the occurrence of this form, but figs. 27, 28 of pl. 18, in her work on *Chlorococcum humicola* ('19b), are identical in appearance with the forms observed in the present cultures, and possibly represent the same organism. It is noteworthy that this species has the record for depth, having been obtained at a depth of more than 9 feet.

Species C

This form is apparently another species of *Chlamydomonas*. It was only observed with certainty in a single culture in which it occurred pure. It may perhaps have been present in other instances, being possibly overlooked in the confusing mixture of other forms. The macroscopic appearance of the culture is very different from that of the preceding species, for the alga, instead of forming a large gelatinous stratum, as in Species B, produces small tough green flakes. Microscopically, the cells are somewhat rounded, possess a distinct pyrenoid, and are arranged in gelatinous clumps of greater or smaller size, though never forming such a large expansion, or possessing so much mucilaginous material as Species B. A slight change in the external conditions induces the development of cilia, as before, and the cells become motile and swim out of the gelatinous stratum. The motile cell is a little stouter than in Species B, reaching a length of 5–9 μ and a breadth of 3.5–6 μ. The chloroplast is not so distinctly bell-shaped; it leaves a larger and more irregularly shaped space clear at the anterior end, and the stigma is very minute and difficult to find, and also, when visible, is more anterior in position. The chief differences between the two species are to be found in the chloroplast and stigma and in the macroscopic nature of the colonies. It is possible that the *Protococcus*-like stages referred to above may belong to this species.

Species D

In many of the cultures gelatinous masses were frequently observed among the other forms, in which the cells embedded in the gelatinous stratum possessed the stigma and other characters of motile cells. The slightly changed conditions resulting from the removal of the sample from the large culture vessel, and its examination on a slide almost invariably induced the small cells to become motile. Then, in the earlier examination of the cultures, these motile cells were always observed to unite in pairs, producing a rounded zygote. An attempt was made, but without success, to follow the development of the zygote, and unfortunately the alga never occurred alone, or in sufficient quantity to make its isolation possible. In older cultures a similar form was frequently observed which agreed in size and in its conspicuous stigma, but the contents were so obscured by the presence of large starch grains that other cytological comparisons were impossible. Although in these older cultures the cells readily became motile as before, conjugation was not observed to occur.

The motile cells of this alga are smaller and more rounded than in the two preceding forms. They are $3\frac{1}{2}$–5 μ in diameter, and only slightly longer than broad. The chloroplast is not particularly massive, covering only a part of the external wall, and contains a pyrenoid which is usually quite conspicuous. The stigma is distinctly visible and the cilia are somewhat longer than the body length. The form has been observed as far down as 4 feet.

Species E

This is an additional species of *Chlamydomonas* which was not nearly such a constant constituent of the soil as some of the others, or at least it never occurred in quantities large enough to be conspicuous, although it may sometimes have been present as isolated individuals. It was never observed in a motile condition, but that it is normally a motile organism and probably a species of *Chlamydomonas* seem to be undoubted facts. It was readily distinguished from all other similar forms by its size, reaching a length of 12–21 μ and a breadth of 10–12 μ. The cells were usually broadly oval and occurred in most cases in the palmelloid form, each cell surrounded by its own distinct gelatinous envelope

which might reach a thickness of 10 μ, and aggregated in larger or smaller clumps. Very often active cell division seemed to have been taking place, for 2–8 smaller individuals were sometimes seen crowded together in the same envelope. In the majority of the larger individuals it could be clearly recognized that the alga was normally a ciliated organism by the differentiation of the cell contents, a clear apical region being easily distinguished. Apart from this, too much reserve food, both starch and oil, was usually present for the cytological structure to be clearly seen. The lowest depth at which it was found is 4 feet.

Species F

There was some doubt at first whether this organism is really an alga or a large bacterium, but the balance of evidence seems to be in favor of its being an alga, most probably of the genus *Stichococcus*. The cells are very minute, oblong and angular, 1½–2 μ broad and 4–5 μ long. They are distinctly, though faintly, green in color and seemed at first to have homogeneous contents. The higher magnification of the oil immersion showed, however, that in some individuals a clear space could be recognized either at one or both ends or else along the lateral margin. This seems to indicate that there is a chloroplast in the form of an extensive parietal plate. There is no pyrenoid, and very little blackening with the addition of iodine. The bacillus-like form of the organism at once distinguishes it from *Stichococcus bacillaris*. Information concerning its reproduction is to be desired before its exact affinities can be decided. The species was observed only in one or two isolated cultures.

Species G

This is one of the several forms peculiar to series D, in a number of samples of which it occurred, including some from a depth of more than 6 feet. The cells are isolated and spherical, 9–13 μ in diameter, reaching in exceptional cases 19 μ. There was usually a single bright green chloroplast, occasionally two, but neither pyrenoids nor starch were present, their place being taken by oil. Reproduction occurred by the formation of aplanospores which were produced in large numbers within a mother cell. These

small aplanospores gradually increase in size until they reach the dimensions of the ordinary vegetative cells. Until the swarmspores of the alga have been obtained, its systematic position cannot be stated.

Species H

In two cultures from a depth of 3 feet, brownish patches appeared between the sand and the glass at the bottom of the cultures, which were at first thought to be due to diatoms, but which proved, on examination, to consist of minute organisms probably of a flagellate nature. The tiny cells were 2.5–3 μ wide by 4.5–5 μ long, and had a bell-shaped brownish green chromatophore lining the greater part of the outer membrane. There was no pyrenoid. The organisms were not observed in the motile condition, and nothing is known of their cilia. There was always a very conspicuous projecting stigma, however, which indicates that they are normally motile.

Species I

This puzzling form occurred in only two cultures, one from a depth of 5 feet 8 inches and the other 8 feet 7 inches. It consists of small oval cells 4–7.5 μ long and 3–5 μ wide. There is a single parietal chloroplast which often does not cover the entire wall, and in which a conspicuous pyrenoid is embedded. Multiplication takes place by the formation of 2–16 aplanospores within a mother cell. Motile stages were not observed, and there is no tendency to the formation of gelatinous colonies.

Species J

? *Ankistrodesmus Pfitzeri* (Schröder) West, Brit. Freshw. Alg. 224. *f. 94 G, H.* 1904.

In association with the preceding species in culture 62 at a depth of 5 feet 8 inches were conspicuous elongated cells. In form they seemed to be very similar to *Ankistrodesmus Pfitzeri*, but they were somewhat smaller and perhaps also a little broader in proportion. The cells were about 10 μ long and 3 μ wide, and there was a parietal chloroplast but no pyrenoid. *A. Pfitzeri* is usually stated to occur in gelatinous colonies, but in this culture the cells, although tending to adhere to each other and therefore

being surrounded by some gelatinous material, did not occur in well-defined colonies. Unfortunately, the species did not persist for very long in the culture but was gradually crowded out by Species I.

Moss Protonema

Moss Protonema was observed in only one culture, at a depth of 6 inches. It can therefore be assumed that normally the filaments do not descend to any great depth as do some algae, but are comparatively superficial in their growth.

TABLE I

(Series A)

SAMPLES TAKEN NOVEMBER 18, 1922, BETWEEN THE EXPERIMENTAL GREENHOUSE AND THE OLD RESIDENCE

½	Number of culture	1a	1b	2a
	Depth	12 inches	12 inches	18 inches
	Date of examination	Mar. 1923 Apr. 1923 Nov. 1923 Apr. 1924	Feb. 1923 Mar. 1923 Apr. 1923 Nov. 1923 Apr. 1924	Feb. 1923 Mar. 1923 Apr. 1923 Apr. 1924
Chlorococcum humicola	×	××	×	×
Chlorochytrium paradoxum	×			
Protosiphon botryoides				
Chlorella sp.		×××		
Botrydiopsis arhiza				
Monocilia viridis				
Stichococcus bacillaris				
Stichococcus scopulinus		××		
Protococcus-like colonies				
Chlamydomonas (unidentified)	?		?	?
Species A	?	?	?	
Species B	?	×		
Species D				
Species E				
Species F		?		
Nostoc muscorum		×		
Nostoc commune		×		
Nostoc sp.			×××	
Lyngbya subtilis				
Phormidium sp.			×	
Navicula atemoides	×	××	×	
Navicula mutica		×	×	
Hantzschia amphioxys				

TABLE I *(Continued)*

Number of culture	Depth	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Monocilia viridis*	*Stichococcus bacillaris*	*Stichococcus scopulinus*	*Protococcus*-like colonies	*Chlamydomonas* (unidentified)	Species A	Species B	Species D	Species E	Species F	*Nostoc muscorum*	*Nostoc commune*	*Nostoc* sp.	*Lyngbya subtilis*	*Phormidium* sp.	*Navicula atemoides*	*Navicula mutica*	*Hantzschia amphioxys*
2b	18 inches	Mar. 1923	×				×					× ×													
		Apr. 1923	×																						
		Nov. 1923	×																						
		Apr. 1924	×	×			×	×				×		×											
3	2 feet	Apr. 1923	×																						
		Nov. 1923	×																						
		Apr. 1924	×			×																			
4	2 ft. 6 in.	Mar. 1923	×	×					?			×					×								
		Apr. 1923	×	×								×													
		Nov. 1923	×										×		×										
		Mar. 1924	×										×		×						×				
		Apr. 1924	×										×								×				
5	3 feet	Mar. 1923		×		×																	×		
		Apr. 1923		×																			×		
		Nov. 1923		×											×								×		
		Apr. 1924		×											×								×		
6	3 ft. 6 in.	Feb. 1923		×				×																	
		Apr. 1923					×	×				×													
		Nov. 1923	×			×									×										
		Apr. 1924	×			×					×														
7	4 feet	Feb. 1923			×							×													
		Apr. 1923	×		×	×						×													
		Nov. 1923	×	×	×	×	×																		
		Apr. 1924	×	×		×																			
8	5 feet	Nov. 1923				×																			
		Apr. 1923				×																			

TABLE II

(Series B)

SAMPLES TAKEN APRIL 19, 1923, NORTHWEST OF CENTRAL LILY POND, MAIN ENTRANCE

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Ulothrix subtilis*	*Characiopsis minuta*	*Chlamydomonas* (unidentified)	Species A	Species B	Species D	Species E	*Protococcus*-like stage	*Gloeocystis*-like stage	*Nostoc* sp.	*Navicula atemoides*	*Navicula mutica*	*Nitzschia palea*	*Hantzschia amphioxys*
26a	1 ft. 6 in.	May 1923	×								×											
		Jan. 1924	×	?	×	×		×						×								
		Apr. 1924	×	×		×		×						×								
26b	1 ft. 6 in.	May 1923	×								×											
		Jan. 1924	×		×																	
		Apr. 1924	×	×				×														
29a	1 ft. 6 in.	May 1923	×		×								×		×				×			
		Feb. 1924	×		×								×		×	×			×			
		Apr. 1924	×		×								×		×							
29b	1 ft. 6 in.	May 1923	×		×						×								×			
		Feb. 1924	×		×	×			×			×							×			
		Apr. 1924	×	?		×			×		×	×						×	×	×		
25a	2 ft. 3 in.	May 1923	×								×											
		Jan. 1924	×																×			
		Apr. 1924	×	?		×													×			
25b	2 ft. 3 in.	May 1923	×								×											
		Jan. 1924	×	×													×		×			
		Apr. 1924	×							×						×	×		×			
24a	2 ft. 6 in.	May 1923	×		×						×								×			×
		Nov. 1923	×		×									×					×			×
		Mar. 1924	×	?	?	×								×								
		Apr. 1924		?	×	×										×			×			
24b	2 ft. 6 in.	May 1923	×		×						×								×			
		Dec. 1923	×		×									×					×			
		Apr. 1924	×											×					×			
27a	2 ft. 6 in.	May 1923	×		×						×											
		Jan. 1924	×		×									×					×			
		Apr. 1924	×	?	×									×								
27b	2 ft. 6 in.	May 1923	×		×																	
		Jan. 1924	×	?	×						×											
		Apr. 1924	×	?	×									×								

TABLE II (*Continued*)

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Ulothrix subtilis*	*Characiopsis minuta*	*Chlamydomonas* (unidentified)	Species A	Species B	Species D	Species E	*Protococcus*-like stage	*Gloeocystis*-like stage	*Nostoc* sp.	*Navicula atemoides*	*Navicula mutica*	*Nitzschia palea*	*Hantzschia amphioxys*
23a	3 feet	May 1923	×		×						×								×			
		Nov. 1923	×	?	×	×					×								×			
		Apr. 1924	×	?	×	×					×								×			
23b	3 feet	May 1923	×		×						×								×			
		Nov. 1923	×		×						×								×			
		Apr. 1924	×		×	×					×								×			
28a	3 feet	May 1923	×		×						×								×			
		Feb. 1924	×	×	×	×								×					×			
		Apr. 1924	×		×	×													×			
28b	3 feet	May 1923	×		×						×								×			
		Feb. 1924	×	?	×												×		×			
		Apr. 1924	×	?	×	×							×						×			
22a	3 ft. 6 in.	May 1923	×		×																	
		Nov. 1923	×	?	×						×											
		Apr. 1924	×			×							×									
22b	3 ft. 6 in.	May 1923	×																			
		Nov. 1923	×		×	×					×											
		Apr. 1924	×	?	×	×							×									
21a	4 feet	May 1923	×		×																	
		Nov. 1923	×	?	×	×					×											
		Apr. 1924	×	??	×	×										×						
21b	4 feet	May 1923	×	×	×																	
		Nov. 1923	×	?	×	×								×								
		Apr. 1924	×	?	×	×					×											
30	4 feet	May 1923	×		×						×											
		Feb. 1924				×										×		×		×		
		Apr. 1924	×		×	×	×													×		

TABLE III

(Series C)

SAMPLES TAKEN JUNE, 1923, SOUTH OF GRADUATE LABORATORY

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Trochiscia* sp.	*Chlamydomonas* (unidentified)	*Dactylococcus* sp.	Species A	Species B	Species D	Species E	*Gloeocystis*-like stage	*Nostoc commune*	*Nostoc* sp.	*Phormidium tenue*	*Phormidium molle*	*Navicula atemoides*	*Nitzschia palea*
50	1 foot	Feb. 1924	×			×							×				××		×			
		Apr. 1924	×																			
49	1 ft. 4 in.	Oct. 1923	×		×××																	
		Feb. 1924	××										××				×					
		Apr. 1924	×												×							
48a	1 ft. 8 in.	Oct. 1923	×		×××																	
		Feb. 1924	××	?		×				××			×				××		×			
		Apr. 1924	×	?	×										×							
48b	1 ft. 8 in.	Oct. 1923	×		×																	
		Feb. 1924	×		×																	
		Apr. 1924	×		×	×																
47	2 feet	Oct. 1923	×																			
		Feb. 1924	×	×								×										
		Apr. 1924	×	×							×	×										
46	2 ft. 3 in.	Oct. 1923	×		×			×														
		Feb. 1924	×		×			×		×												
		Apr. 1924	×		×																×	
45a	2 ft. 6 in.	Oct. 1923	×		×			×														
		Feb. 1924	×		×			×														
		Apr. 1924	×					×														
45b	2 ft. 6 in.	Oct. 1923	×		×																	
		Feb. 1924	×		×						×				×							
		Apr. 1924	×								×				×							
44a	3 feet	Oct. 1923	×																			
		Feb. 1924	×	×							×											
		Apr. 1924	×	×							×		×								×	
44b	3 feet	Oct. 1923	×		×																	
		Feb. 1924	×		×																	
		Apr. 1924	×		×																	
43a	3 ft. 1 in.	Oct. 1923	×	?	×																	
		Feb. 1924	×	?	×	×																
		Apr. 1924	×	?	×	×																

TABLE III (*Continued*)

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Trochiscia* sp.	*Chlamydomonas* (unidentified)	*Dactylococcus* sp.	Species A	Species B	Species D	Species E	*Gloeocystis*-like stage	*Nostoc commune*	*Nostoc* sp.	*Phormidium tenue*	*Phormidium molle*	*Navicula atemoides*	*Nitzschia palea*
43b	3 ft. 1 in.	Oct. 1923	×			×																
		Feb. 1924	×			×																
		Apr. 1924	×			×							×									
43c	3 ft. 1 in.	Oct. 1923																				
		Feb. 1924					×															
		Apr. 1924					×															
42a	3 ft. 3 in.	Oct. 1923				×																
		Jan. 1924				×							×									
		Apr. 1924				×							×									
42b	3 ft. 3 in.	Oct. 1923	×																			
		Feb. 1924	×	?												×						
		Apr. 1924	×	×		×					×					×					×	
42c	3 ft. 3 in.	Oct. 1923										×										
		Feb. 1924										×										
		Apr. 1924										×										
41a	3 ft. 6 in.	Oct. 1923	×																			
		Feb. 1924	×										×	×								
		Apr. 1924	×										×									
41b	3 ft. 6 in.	Oct. 1923	×																			
		Feb. 1924	×																		×	
		Apr. 1924	×																		×	
41c	3 ft. 6 in.	Oct. 1923	×																			
		Feb. 1924											×									
		Apr. 1924											×									
40a	3 ft. 9 in.	Produced no growth																				
40b	3 ft. 9 in.	Oct. 1923	×																			
		Feb. 1924	×							×												
		Apr. 1924	×																			
40c	3 ft. 9 in.	Oct. 1923	×																			
		Feb. 1924	×				×				×											
		Apr. 1924	×				×			×	×											

TABLE IV

(Series D)

SAMPLES TAKEN IN "LOWER" GARDEN BEHIND GREENHOUSES, JANUARY 14, 1924.
(TEMP. 22° F.)

Number of culture	Depth of sample	Weight of inoculum	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Stichococcus bacillaris*	*S. scopulinus*	*Ulothrix subtilis*	*Uronema confervicola*	Species B	Species C	Species E	Species F	Species G	Species H	Species I	Species J	Moss Protonema	*Nostoc communutum*	*Oscillatoria amphibia*	*Navicula atemoides*	*Nitzschia palea*
77	6 in.	5 gm.	Feb. 1924 Apr. 1924	× ×	× ×				× ×		× ×	× ×				×						×				
76i	12 in.	.01 gm.	Mar. 1924 Apr. 1924	× ×					× ×																	
76h	12 in.	.02 gm.	Mar. 1924 Apr. 1924	× ×					× ×																	
76g	12 in.	.05 gm.	Mar. 1924 Apr. 1924	× ×					× ×					×												
76a	12 in.	.1 gm.	Feb. 1924 Apr. 1924	× ×			× ×		× ×																×	
76b	12 in.	.2 gm.	Feb. 1924 Apr. 1924	× ×			× ×		× ×					×												
76c	12 in.	.5 gm.	Feb. 1924 Apr. 1924	× ×					× ×					×												
76d	12 in.	1 gm.	Feb. 1924 Apr. 1924	×			× ×		 ×					× ×												
76e	12 in.	2 gm.	Feb. 1924 Apr. 1924	× ×		×	× ×		× ×		× ×			× ×												
76f	12 in.	5 gm.	Feb. 1924 Apr. 1924	× ×		×	× ×			×	× ×					× ×	×								×	
75	1 ft. 6 in.	5 gm.	Mar. 1924 Apr. 1924	× ×			× ×		× ×			× ×		×												
74a	2 feet	.1 gm.	Mar. 1924 Apr. 1924															× ×								
74b	2 feet	.2 gm.	Mar. 1924 Apr. 1924	× ×			 ×																			
74c	2 feet	.5 gm.	Mar. 1924 Apr. 1924	× ×	×									?												
74d	2 feet	1 gm.	Mar. 1924 Apr. 1924	× ×			× ×		× ×									×								
74e	2 feet	2 gm.	Mar. 1924 Apr. 1924	× ×					× ×					× ×											×	
74f	2 feet	5 gm.	Mar. 1924 Apr. 1924	× ×	× ×	×	× ×		× ×					×		×										

TABLE IV (*Continued*)

Number of culture	Depth of sample	Weight of inoculum	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arrhiza*	*Trochiscia reticularis*	*Stichococcus bacillaris*	*S. scopulinus*	*Ulothrix subtilis*	*Uronema confervicola*	Species B	Species C	Species E	Species F	Species G	Species H	Species I	Species J	Moss Protonema	*Nostoc communum*	*Oscillatoria amphibia*	*Navicula atemoides*	*Nitzschia palea*
73	2 ft. 6 in.	5 gm.	Mar. 1924 Apr. 1924	× ×			× ×		× ×	×				×			×								×	—
72	2 ft. 9 in.	5 gm.	Mar. 1924 Apr. 1924	× ×		× ×	× ×	×	× ×																× ×	—
71a	3 feet	.1 gm.	Mar. 1924 Apr. 1924	×										× ×												—
71b	3 feet	.2 gm.	Mar. 1924 Apr. 1924												× ×											—
71c	3 feet	.5 gm.	Mar. 1294 Apr. 1924	× ×										× ×												—
71d	3 feet	1 gm.	Mar. 1924 Apr. 1924				× ×		×			×	×	× ×							×					—
71e	3 feet	2 gm.	Mar. 1924 Apr. 1924	× ×	 ×								×	× ×		× ×					×					—
71f	3 feet	5 gm.	Mar. 1924 Apr. 1924	× ×	 ×		× ×		× ×			× ×		× ×		× ×										—
70a	3 ft. 6 in.	5 gm.	Mar. 1924 Apr. 1924																				× ×			—
70b	3 ft. 6 in.	over 5 gm.	Mar. 1924 Apr. 1924	× ×	× ×	 ×		× ×							? ?											—
69a, b, c	4 ft. 2 in.	.5 gm., 1 gm., and 2 gm. respectively	Produced no growth																							
69d	4 ft. 2 in.	5 gm.	Mar. 1924 Apr. 1924															× ×								—
69e	4 ft. 2 in.	over 5 gm.	Mar. 1924 Apr. 1924	× ×										× ×				× ×								×
68a, b	5 ft. 5 in.	.5 gm. and 1 gm. resp.	July 1924	×																						—
68c	5 ft. 5 in.	2 gm.	Mar. 1924 Apr. 1924																						× ×	—
68d	5 ft. 5 in.	5 gm.	Mar. 1924 Apr. 1924	× ?																						—

TABLE IV (*Continued*)

Number of culture	Depth of sample	Weight of inoculum	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Stichococcus bacillaris*	*S. scopulinus*	*Ulothrix subtilis*	*Uronema confervicola*	Species B	Species C	Species E	Species F	Species G	Species H	Species I	Species J	Moss Protonema	*Nostoc communutum*	*Oscillatoria amphibia*	*Navicula atemoides*	*Nitzschia palea*
68e	5 ft. 5 in.	over 5 gm.	Mar. 1924 Apr. 1924	× ×										×												
67a	5 ft. 8 in.	.5 gm.	May 7 1924																		×					
67b -c	5 ft. 8 in.	1 gm., 2 gm., 5 gm. and over, respectively		Produced no growth																						
66	6 ft. 4 in.	over 5 gm.		Produced no growth																						
65a–c	6 ft. 10 in.	.5 gm., 1 gm., and 2 gm. respectively		Produced no growth																						
65d	6 ft. 10 in.	5 gm.	Mar. 1924 Apr. 1924												× ×											
65e	6 ft. 10 in.	over 5 gm.		Produced no growth																						
64	7 ft. 6 in.	over 5 gm.		Produced no growth																						
63a–d	8 ft. 2 in.	.5 gm., 1 gm., 2 gm. and 5 gm. respectively		Produced no growth																						
63e	8 ft. 2 in.	over 5 gm.	Apr. 1924																		×					
62	8 ft. 7 in.	over 5 gm.	Apr. 1924																		×	×				
61a–c	9 ft. 1 in.	1 gm., 2 gm., 5 gm. respectively		Produced no growth																						
61d	9 ft. 1 in.	over 5 gm.	Mar. 1924	×																						
60a–c	9 ft. 5 in.	1 gm., 2 gm., and 5 gm. respectively.		Produced no growth																						

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